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Membrane protein detection

Shao-Cong Sun xiaofei zhou

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An abbreviated version of this protocol was published in Nat Immunol in Jun 2019

The deubiquitinase Otub1 controls the activation of CD8 T cells and NK cells by regulating IL-15-mediated priming

DOI: 10.1038/s41590-019-0405-2

Detailed protocol

Procedure for membrane protein extraction from lymphocytes

Materials and reagents

1. Splenocytes from mouse
2. PBS (Corning, catalog number:21-040-CV)
3. RBC lysis buffer (sigma, catalog number: R7767-100ml)
4. Anti-CD4 microbeads (130-117-043), anti-CD8 microbeads (130-117-044), NK cell isolation kit (130-115-818) from Miltenyi Biotec
5. Mem-Per Plus membrane protein extraction kit (Thermo fisher Scientific, catalog number: 89842)
6. Protease and phosphatase inhibitor (Themo fisher, catalog number :78440)
7. Protein assay dye reagent concentrate (Bio-Rad, catalog number: #5000006)
8. a-tubulin (cell signaling, catalog number:2144); IGF1Rb (cell signaling, catalog number:3018)
9.

Equipment:

1. Centrifuge 5424R (Eppendorf)
2. Epoch Microplate Spectrophotometer(Biotek)

To extract the membrane and cytosol proteins, we used the mem-Per plus membrane protein extraction kit (Cat. 89842) from thermo fisher with modification.
We firstly isolate mouse CD4, CD8 from pooled spleens and lymph nodes, isolate mouse NK cells from pooled spleens by using microbeads.

Procedure for cytosolic and membrane protein isolation:

1. Harvest 10×10^6 CD4, CD8, NK cells by centrifugation at 300 × g for 5 minutes in 2ml centrifuge tube. Wash harvested cell pellet with 2mL of Cell Wash Solution and centrifuge at 300 × g for 5 minutes at 4^{o}C.
2. Carefully remove and discard the supernatant. Resuspend the cells in 2mL of Cell Wash Solution, centrifuge at 300 × g for 5 minutes at 4^{o}C and discard supernatant again.
3. Add 60ul of Permeabilization Buffer with Protease and phosphatase inhibitor to the cell pellet. Vortex briefly to obtain a homogeneous cell suspension. Incubate 10 minutes at 4^{o}C with constant mixing.
4. Centrifuge permeabilized cells for 15 minutes at 16,000 × g at 4^{o}C. Carefully remove the supernatant containing cytoslic proteins and transfer to a new 1.5ml tube.
5. Add 500ul Permeabilization buffer Protease and phosphatase inhibitor to cell pellet, pipetting up and down. Centrifuge the pellet for 5 minutes at 16,000 × g at 4^{o}C. Carefully remove the supernatant completely. (wash step, optional)
6. Add 40ul of solubilization buffer with Protease and phosphatase inhibitor and resuspend by pipetting up and down. Incubate tubes at 4^{o}C for 30 minutes with constant mixing.
7. Centrifuge tube at 16,000x g for 15 minutes at 4^{o}C. transfer supernatant containing solubilized membrane and membrane associated proteins to a new tube.
8. Measure protein concentration by using the Protein assay dye reagent at 595nm.
9. Add 2x loading dye to protein, boil the sample for 5 minutes at 100^{o}C and run westblot immediately, or store protein samples aliquots at -20 ^{o}C for future use. a-tubulin and IGF1Rb serve as cytosolic and membrane fraction marker separately.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sun, S. and zhou, x. (2021). Membrane protein detection. Bio-protocol Preprint. bio-protocol.org/prep1225.
2. Zhou, X., Yu, J., Cheng, X., Zhao, B., Manyam, G. C., Zhang, L., Schluns, K., Li, P., Wang, J. and Sun, S.(2019). The deubiquitinase Otub1 controls the activation of CD8 T cells and NK cells by regulating IL-15-mediated priming. Nat Immunol 20(7). DOI: 10.1038/s41590-019-0405-2